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Acute Toxic Effects of Petroleum Refinery Wastewaters on Redear Sunfish

Robert S Kerr Environmental Research Lab, Ada, Okla

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Bioassays were performed using redear sunfish (Lepomis microlophus) as test organisms. Twenty-four hour 50 percent tolerance limits (TL_{50}) of the various wastewaters are compared with results of chemical analyses performed during the same study. Toxicity varied considerably both between refineries and for waste streams from within a single refinery. Results of these analyses and observed behavioral symptoms of distressed fish revealed that ammonia, sulfides, and phenolics, alone or in combination, were major contributors to toxicity exerted in most samples. Three refineries had samples which were more toxic than anticipated based on results of chemical analyses, indicating the presence of other toxic compounds in unknown quantities; e.g., various hydrocarbons.

Results of bioassays of samples from various wastewater treatment stages of five refineries with activated sludge treatment systems revealed a decrease in toxicity as the degree of treatment increased indicating a reduction in concentration of contributing biodegradeable toxicants.

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ABSTRACT

Static bioassays of 24 hours' duration were performed on samples of wastewaters provided by 22 domestic petroleum refiners. These wastewaters represent three types of water discharges prevalent to this industry: process wastewaters prior to dilution with other streams; API separator effluents which are a conjugate of various streams within a refinery; and wastewaters following treatment by activated sludge systems.

Bioassays were performed using redear sunfish (Lepomis microlophus) as test organisms. Selection of this species was due to its being a member of the important freshwater family, Centrarchidae, its widespread distribution, and the availability of a plentiful supply with a controlled background.

Twenty-four hour 50 percent tolerance limits (TL_{50}) of the various wastewaters are compared with results of chemical analyses performed during the same study. Toxicity varied considerably both between refineries and for waste streams from within a single refinery. Results of these analyses and observed behavioral symptoms of distressed fish revealed that ammonia, sulfides, and phenolics, alone or in combination, were major contributors to toxicity exerted in most samples. Three refineries had samples which were more toxic than anticipated based on results of chemical analyses, indicating the presence of other toxic compounds in unknown quantities; e.g., various hydrocarbons.

Results of bioassays of samples from various wastewater treatment stages of five refineries with activated sludge treatment systems revealed a decrease in toxicity as the degree of treatment increased indicating a reduction in concentration of contributing biodegradeable toxicants.

This report was submitted in fulfillment of ROAP 21 AZP, Task 050 by the Robert S. Kerr Environmental Research Laboratory of the U. S. Environmental Protection Agency. Work was completed as of January 1974.

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SECTION I

CONCLUSIONS

Short-term static bioassays of 24-hours' duration can be an effective tool for screening industrial process wastewaters to locate sources of toxic agents; these tests can also be used to evaluate effectiveness of industrial waste treatment processes.

Static bioassays cannot be used to obtain reliable 50 percent tolerance limit (TL_{50}) values for low toxicity wastes that exert a high oxygen demand although a range can often be established by an experienced observer. Dissolved oxygen (DO) usually becomes critical at about 12 hours; low DO plays a major role in a mortaility after this time. Activated sludge treatment processes tend to reduce the oxygen demand beyond the critical stage.

Raw wastewaters from different oil refineries vary greatly in their toxic characteristics; wastewaters from different processes within a single refinery also vary greatly in their toxic characteristics. Toxicity of oil refinery wastewaters varies considerably at different treatment stages in the activated sludge process; toxicity appears to decrease following each stage of treatment.

Toxicity of oil refinery wastewaters cannot always be predicted from results of chemical analyses; the toxic effect of the waste is dependent on the synergistic or antagonistic activity of toxicants present.

The most common toxic constituents of untreated oil refinery wastewaters are: ammonia, sulfides, phenolic compounds, and cyanides. Raw wastewaters also may contain other toxic compounds including various hydrocarbons.

Due to the volatile and unstable nature of some toxic components of oil refinery wastewaters which may have led to a reduction in concentrations during sample transportation, storage, and handling, TL_{50} values obtained during these tests may be higher than the actual value.

Acute toxic effects of raw wastewaters from oil refineries are generally exerted within the first 12 hours of the static test; therefore, a 24-hour test will provide good positive results under static conditions. If samples containing toxic compounds, other than those mentioned above, toxic effects may be exerted over an extended period. Tests should then be continued for at least 48 hours to obtain more positive results.

Toxicity of the final clarifier effluent from oil refineries with activated sludge treatment systems is dependent on toxic constituents present in the influent and their concentrations. Results of chemical analyses conducted during these tests indicate that concentrations of most toxicants other than ammonia are reduced by activated sludge treatment systems and at least a four-fold decrease in toxicity can be expected after treatment.

Although activated sludge treatment systems have very little effect on ammonia concentrations, the toxic effect of ammonia is lessened due to a decrease in pH of the treated effluent.

SECTION II

RECOMMENDATIONS

With the many and varied processes employed by oil refineries in the refining of crude oil, the results of this study and others indicate the need to further document the effect of treated refinery wastewaters and individual wastewater constituents on fish and other aquatic life in receiving waters under a wide range of environmental conditions.

This project was conducted to determine short-term acute toxic effects of refinery wastewaters on a single fish species. A research study to investigate long-term cumulative effects of treated refinery wastewaters on fish and other aquatic life should be conducted.

The oil refining industry should be encouraged to use short-term static bioassays to assist in their pollution control effort. Some of the ways in which this type of bioassays can be used are:

1. Acute toxicity of final effluents can be determined.
2. Toxicity of individual process wastewaters can be determined. These effluents, if highly toxic, may be modified, eliminated, or treated.
3. Location of processes which contribute high toxicity wastewaters make possible alternative methods of controlling the potential problem. The smaller quantities of wastewater may be pretreated to reduce toxicity or stored with regulated release into the refinery wastewater treatment system.
4. Leaks, spills, or other losses of toxic compounds into waste streams can be detected, located, and remedied.
5. The effectiveness of treatment systems on removing toxic compounds can be established.

SECTION III

INTRODUCTION

Industrial wastes may be detrimental to aquatic life by direct toxic action or by altering natural qualities of the receiving water. Petroleum refinery wastewaters can be characterized as having a high biochemical oxygen demand (BOD) and significant toxicity.[1] Adverse effects from acute concentrations of refinery effluents during several days of exposure have been reported.[2] Experiments with five species of fish have shown that the emulsified effluent from American Petroleum Institute's (API) gravity oil separators is toxic to fish unless diluted or otherwise treated.[3] Treatment of refinery effluents to reduce toxicity also results in improvement in other objectionable characteristics including oil content, oxygen demand, chemical composition, turbidity, and odor.[4]

Products and wastes from oil refineries are extremely complex in their composition and their effects on water quality. While it is recognized that each particular oil refinery poses its individual problems, certain pollutants are common to all refineries. Process wastewaters are high in phenolics, ammonia, and hydrogen sulfide and pose the primary water pollution problem.[5] All of these compounds are relatively high in toxicity to aquatic life. In addition to these common constituents, process wastewaters may contain various hydrocarbons toxic to aquatic life.[6, 7] Findings during a cooperative American Petroleum Institute/ Environmental Protection Agency (API/EPA) waste characterization study of petroleum refinery wastewaters conducted at the Robert S. Kerr Environmental Research Laboratory (RSKERL) concurrently with this

project indicate that these wastewaters may contain a large number of substances which if discharged without treatment into receiving waters could pose a serious threat to fish and other aquatic life.[8] It was also found, however, that refinery treatment processes substantially reduce the acute toxic effect of these substances.

Refinery wastewaters often contain a number of these different toxicants which, when mixed or under the influence of other characteristics of the effluent or receiving water, may produce an entirely different toxicity level from that of the pure components. Results of chemical analyses for possible toxic components compared with toxicity levels reported in the literature for each particular component can not always be expected to produce satisfactory answers.

The concentration of these pollutants in a particular waste stream is dependent on the process from which the stream flows as well as in-process procedures for reduction of a potential troublesome pollutant. The concentration in the final effluent is dependent on the type and efficiency of treatment to which the wastewater is subjected.

The use of some type of bioassay to determine the toxicity of a complex wastewater can be the most effective and accurate method of assessing potential danger. The bioassay provides a direct satisfactory method for evaluating acute toxicity of chemically complex wastes. Toxicity determinations rather than complete reliance on BOD and chemical determinations are necessary for the safe disposal of complex wastes.

Two phases of a study of oil refinery wastewaters were conducted at the RSKERL during 1972. During Phase I of the study, samples were collected by EPA and API personnel from in-plant wastewaters at 17 selected refineries located throughout the United States and shipped to the RSKERL for analysis of 28 parameters. A single set of samples was received for each of the refineries. Samples were preserved in tightly capped cubitainers and refrigerated at 4° C until tested. The

number of sample points tested for each refinery ranged from 1 to 13. During Phase II of the study, samples were collected from various stages of the activated sludge treatment process at five selected oil refineries. Samples were collected for 14 consecutive days at each refinery.

On March 28, 1972, a series of 24-hour static bioassays were started at the RSKERL to screen samples collected during Phase I of the study. The purpose of these tests were (1) to evaluate and compare toxicity of in-plant wastewaters of selected oil refineries throughout the United States; (2) to compare actual toxicity results with anticipated toxic action derived from results of chemical analyses of the samples for potential toxicants normally contained in process wastewaters; (3) to check for the presence of unknown toxic compounds; and (4) to observe and catalog behavioral activities of fish exposed to these wastewaters.

On May 10, 1972, a series of 24-hour static bioassays were started on samples collected during Phase II of the study. Two or three bioassays were conducted on composites of the 14 samples collected at each location. The purpose of these tests were (1) to evaluate and compare toxicity at different stages of treatment of selected refineries with activated sludge treatment systems; and (2) to evaluate the efficiency of activated sludge treatment for removal of toxic constituents from refinery wastewaters.

SECTION IV

METHODS

Bioassay procedures used were modified from those described in Standard Methods for the Examination of Water and Wastewater, 13th Edition.[9] Modifications were necessary because of the small volume of sample available for use, the number of samples to be tested, and the resources available. Test procedures outlined in this paper can be expected to measure short-term effects only. The long-term effect is not measured. Thus, there may be a cumulative effect of the waste over a longer period of time.

A series of three to five dilutions and a control were set up for each sample or composite tested. Test concentrations were prepared on a percent-by-volume basis in the diluent water. Two, one-gallon glass jars containing three and one-half liters in each or one, five-gallon glass jar containing ten liters were used per dilution. Five test organisms were placed in each jar after the necessary volume of sample was mixed with the diluent water. This gave either ten fish in seven liters per dilution or five fish in ten liters per dilution. The latter was used to try to alleviate problems of low oxygen in some of the tests and to ensure that stress from crowded conditions did not affect the test results.

Test organisms used were redear sunfish (Lepomis microlophus) that were 20-50 millimeters in length and weighing 0.5-1.5 gram each. Selection of this species was due to its being a member of the important family Centrarchidae, its widespread distribution, and the availability of a plentiful supply with a controlled background. This species was easily maintained under laboratory conditions and

proved highly satisfactory for conducting short-term static bioassays. Fish were obtained from the National Fish Hatchery, Tishomingo, Oklahoma, as needed. Fish were held in stainless steel holding tanks at a constant temperature and saturated dissolved oxygen level until used. Only fish that appeared to be in excellent condition were used in the testing.

The dilution range selected for each sample was based on chemical data for that sample compared with reported toxicity values for measured chemical constituents. If fish died in all dilutions for a test, it was assumed that unknown toxic agents were present in the sample and lower series of dilutions were tested until a TL_{50} value was obtained. Logrithmic or geometric series of dilutions were used in all tests.

Dilution water in all tests was tap water from the Ada, Oklahoma, water supply system (pH 8.3-8.7, Hardness 300-350 mg/1 as $CaCO_3$). Use of a common dilution water made it possible to compare toxicity levels of samples from different refineries. Dilution water in each test container was aerated to saturation prior to adding the sample and test organisms. Aeration was <u>not</u> used during the test period because of the volatile nature of many of the constituents of the wastewaters.

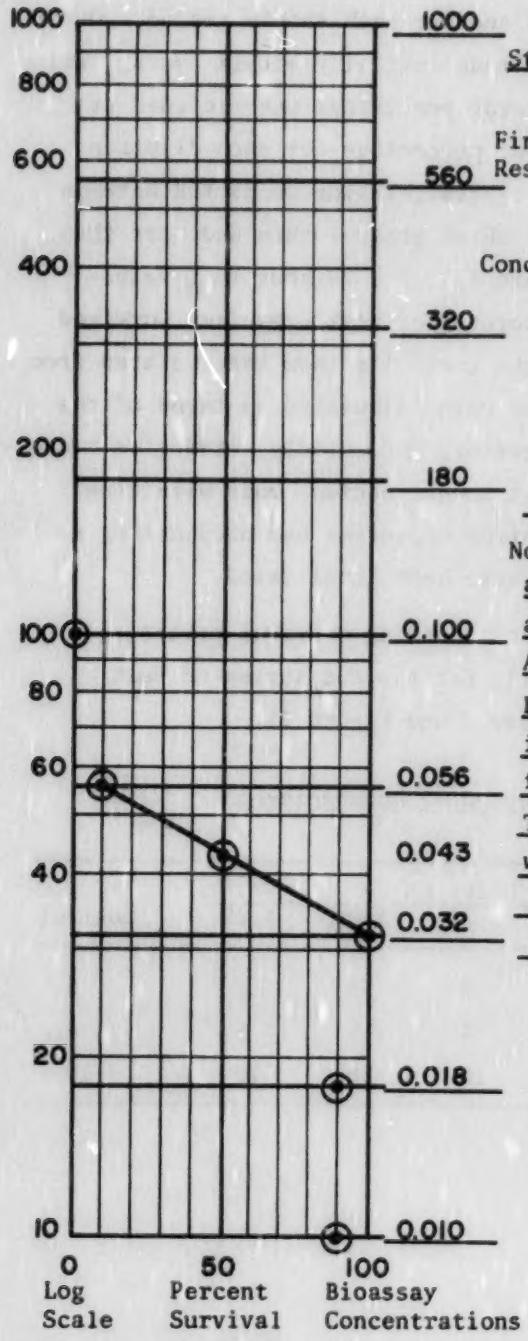
Preliminary tests indicated that most toxicity of oil refinery wastes in static tests is exerted during the first 12 hours. This may be due to the volatile nature of many of the toxic constituents. Personal communication with other scientist who have tested similar wastes confirmed this.[10, 11] Therefore, 24 hours was chosen as the test period. The 24-hour test period tended to eliminate low DO as a contributor to death in latter stages of the tests during Phase I. Low DO during the latter stages was still a problem during Phase II because of the higher waste volumes used in the tests.

Bioassay record sheets were maintained for each sample tested. Observations and selected analysis were made initially at six hours, twelve hours and twenty-four hours. Survival percentage was recorded at each dilution. The 24-hour survival percentage for each dilution was plotted on semi-log paper and a straight line connected between successive dilution percentages at which greater than and less than 50 percent survival occurred (Figure 1). The 24-hour TL_{50} value (percentage dilution at which 50 percent of test organisms survived for 24 hours under conditions of the test) was then interpolated from the straight line. All TL_{50} values were calculated in terms of the initial percentage volume of wastewater; undoubtedly, effective concentrations of the various toxic constituents became lower with time. If more than 10 percent of the control organisms had died during a test, results for that test would have been invalidated.

Within test precision was checked by plotting survival percentages and establishing a TL_{50} individually for the two series of test containers used for each test (Table 1 and Figure 1).

Table 1. WITHIN TEST CHECK OF PRECISION

Test Containers	Dilution					Control
	10%	5.6%	3.2%	1.8%	1.0%	
A	5	5	5	5	5	5
B	5	5	5	5	5	5
Total	10	10	10	10	10	10



Sample No. 6992:RW-3

Starting Date: 3-29-72

Hour: 9:00 a.m.

Final Results: TL_{50}

	Time Intervals		
	A-24	B-24	24 hr
	0.042	0.045	0.043

Concentrations expressed as percent

0.1% → 0.01%

Test species: Redear Sunfish

Temperature range: 21.5°-23°C

Dilution water source:
Ada City Water

Notes: Wild erratic swimming and loss of equilibrium noted at 5 minutes in 0.1% dilution. All fish dead in 30 minutes. Eight fish dead in 2 hours in in 0.056% dilution. Two live fish in obvious distress. Tests continued for 48 hours with no change in TL_{50} value.

John E. Matthews

A-Front row of test containers
B-Back row of test containers

Figure 1. Bioassay record sheet

SECTION V

RESULTS

PHASE I

Bioassays were conducted on 50 samples of in-plant wastewaters from 17 oil refineries during this phase of the study. The 24-hour TL_{50} results are presented in Table 2. Because only a limited amount of each sample was available for use, 24-hour TL_{50} values were not obtained for all samples tested. The 24-hour TL_{50} for those samples exhibiting greater than 50 percent survival in all dilutions tested was reported as greater than the highest dilution tested.

A 24-hour TL_{50} ranging from 0.04%-13.5% was established for 20 samples from eight refineries under the conditions of these tests. Bioassay data for the toxic samples are presented in Appendix A. No 24-hour TL_{50} could be established for the remaining 30 samples. Nine refineries had no samples which exhibited toxicity under these conditions at the dilutions tested.

Three refineries had one or more samples toxic at <0.3%. These same refineries also had at least one other sample toxic at <5%. Three additional refineries had one or more samples toxic at <5%. The remaining two refineries for which a 24-hour TL_{50} was established had single in-plant wastewater streams with a TL_{50} of 7.5% and 13.5%.

Three refineries had one or more samples which were considerably more toxic than indicated by results of chemical analyses. Toxicity of these samples was most likely due to the presence of toxic hydrocarbon compounds. The TL_{50} for one sample was attributed to low DO and one sample to low pH.

Table 2. BIOASSAY RESULTS - PHASE I

Sample	24-hour TL_{50} (% by volume)	Comment
6992 RW-1	>10	
" RW-2	0.042	Same at 48 hours
" RW-3	0.043	Same at 48 hours
" RW-4	>10	
" RW-5	>10	
" RW-6	>18	
" RW-7	>15	
" RW-8	>10	
" RW-9	>18	
" RW-10	0.05	Same at 48 hours
" RW-11	3.8	3.2% at 48 hours
" RW-12	>10	
" RW-13	>10	
59991 RW-1	7.5	
69991 RW-1	13.5	
693 RW-1	0.23[a]	.08% at 36 hours
" RW-2	1.8	
" RW-3	7.5	Low DO
69995 RW-1	>10	
" RW-2	>10	
22 RW-1	>18	
" RW-2	>18	
69994 RW-1	4.5	
" RW-2	1.9	Low pH
6993 RW-1	>10	

Table 2 (Continued). BIOASSAY RESULTS - PHASE I

Sample		24-hour TL_{50} (% by volume)	Comment
595	RW-1	10	
"	RW-2	18	
6995	RW-1	6.5	
"	RW-2	18	
"	RW-3	13	Distress
"	RW-4	13.5	Distress
"	RW-5	4.2	
"	RW-6	7.2	
"	RW-7	14	
"	RW-8	16	
"	RW-9	15	
"	RW-10	18	
92	RW-1	10	
"	RW-2	2.4	
59	RW-1	10	
59993	RW-1	10	
"	RW-2	10	
106	RW-1	18	
56	RW-1	10	
67	RW-1	10	
6996	RW-1	10	
"	RW-2	10	
"	RW-3	4.2	2.4% at 48 hours
"	RW-4	4.2	
"	RW-5	0.05	

[a] Toxicity greater than indicated by results of chemical analyses.

Estimated concentrations at the TL_{50} value were made for common toxic constituents found in refinery wastewaters for purposes of identifying the most likely toxic agent(s) in each sample (Table 3). These estimations were made using results of analyses performed on corresponding water samples. Results upon which these estimations are based are presented in Appendix C. These estimations cannot be assumed to be actual concentrations present at the measured TL_{50}; however, analyses of water samples from some of the bioassay test containers after 24 hours indicate that the estimated values are close enough approximations of the real values to use for the purposes intended.

These projected data along with the actual measurements confirm that ammonia, sulfides, and phenolic compounds are the major contributing toxic constituents present in oil refinery wastewaters. Fourteen of the samples for which a TL_{50} was established during this study contained these constituents individually, or in combination, at concentrations reported in the literature to be toxic to fish and aquatic life.[6, 12, 13] Individual or contributing toxic levels of ammonia was estimated in 12 samples, sulfide in nine samples, and phenolics in eight samples. Five samples did not contain sufficient concentrations of these three constituents, individually or in combination, to account for the toxic level found. It is assumed, therefore, that toxicity of these five samples was due to the presence of unknown toxic compounds, e.g., hydrocarbons.

The behavior of distressed and dying fish is often an indication of the type or category of the agent(s) responsible for death.[6, 12, 13, 14] Continuous observations were made during these tests and the exhibited symptoms noted. Observed symptom were compared with behavioral symptoms reported in the literature for constituents suspected to be present.

During this series of tests, distressed and dying fish exhibited four different symptom patterns:

Table 3. ESTIMATED INITIAL CONCENTRATIONS OF COMMON TOXICANTS AT 24-HOUR TL_{50} PERCENTAGE VOLUME (mg/l)

Refinery/Sample No.		Ammonia	Sulfide	Phenol	Cyanide	pH	TL_{50} % by Vol.
6992	RW-2	1.50	1.67	0.22	0.003	8.6	0.042
"	RW-3	0.10	0.02	6.24	0.007	8.6	0.043
"	RW-10	1.68	1.92	0.25	0.001	8.7	0.05
"	RW-11	3.61	4.90	0.05	0.013	8.5	3.8
59991	RW-1	8.85	6.30	0.57	0.056	8.7	7.5
69991	RW-1	5.40	1.89	0.39	0.19	8.2	13.5
693	RW-1	0.22	0.12	0.15	0.001	8.4	0.23
"	RW-2	3.58	0.20	0.01	0.003	9.1	1.8
69994	RW-1	5.63	0.86	0.20	0.017	8.9	4.5
69995	RW-1	5.27	7.15	0.35	-	9.0	6.5
"	RW-3	0.17	0.05	0.07	-	8.6	13
"	RW-4	0.20	0.12	0.07	-	8.6	13.5
"	RW-5	5.04	3.28	2.60	0.004	8.9	4.2
"	RW-6	0.06	0.01	0.03	-	8.4	7.2
92	RW-2	2.04	0.08	3.36	-	9.0	2.4
6996	RW-3	0.22	0.06	0.13	-	7.8	4.2
"	RW-4	14.7	-	-	-	8.3	4.2
"	RW-5	1.35	1.50	0.21	0.061	8.6	0.05
		Measured Concentrations					
6992	RW-2	2.40	1.00				
693	RW-1			0.20			
"	RW-2	3.55					
69994	RW-1	5.40					
69995	RW-1	5.85					
92	RW-2	3.95		2.71			

1. Wild erratic swimming; loss of equilibrium with periodic spurts; death very shortly with gills swollen (ammonia, low pH, some phenolic compounds).
2. Wild erratic swimming; loss of equilibrium; lethargic condition; highly irritable; death after long period (petrochemicals, phenolics).
3. Some hyperactivity prior to loss of equilibrium; no irritation; state of anaesthesia for long period prior to death; gills swollen (cyanides, phenols, sulfides).
4. Slow circular swimming at surface; irritable; quick spurts from lethargic condition; state of anaesthesia on bottom for long period prior to death; gills swollen (petrochemicals, phenols, cyanides, sulfides).

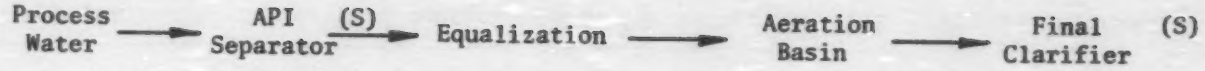
The two most common patterns were those in numbers 1 and 4.

PHASE II

During Phase II, 30 bioassays were conducted on composite samples collected from various stages of the treatment systems of five oil refineries (Figure 2). A total of 12 samples were tested. Two or three bioassays were run on each sample. Results of these toxicity tests are tabulated in Table 4. Low dissolved oxygen in four of the tests prevented establishment of reliable 24-hour TL_{50} values for those samples; hence, 12-hour TL_{50} values for each sample have also been included in Table 4. Bioassay data for each sample are presented in Appendix B. Results of chemical analyses of samples collected from the same sample points concurrent with bioassay samples are presented in Appendix C.

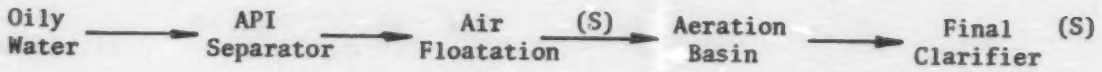
Toxicity values remained relatively constant for different composites from the same sample location. API separator effluent samples from the five refineries exhibited some toxicity. The 24-hour TL_{50} of these samples from the five refineries varied from 4%-50%. Activated

Refinery No. 9973

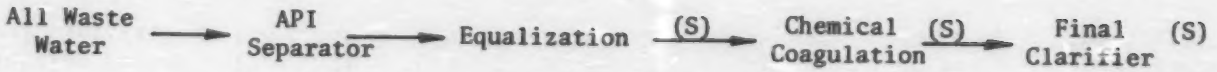


Refinery No. 2115

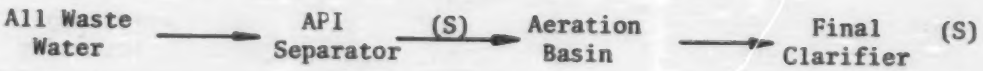
Refinery No. 0288



Refinery No. 6512



Refinery No. 6693



(S) Denotes sample point

Figure 2. Sample points from activated sludge refinery wastewater systems chosen for toxicity tests

Table 4. BIOASSAY RESULTS - PHASE II

Sample Location	Composite Samples 24-hr. TL_{50} A (% Dilution)	B	C	12-hr. TL_{50} A (% Dilution)	B	C	Estimated 24-hr. TL_{50} Range (% Dilution)	Estimated NH_4 Conc. at TL_{50}[a] (mg/l)
Refinery 9973								
API Separator	29[b]	19[b]	14[b]	42	34	41	34-42	3.9 (5.1-3.1)
Final Clarifier	>56	>88	100				100	12.5
	No mortality or stress							
Refinery 2115								
API Separator	4.2	4.2	3.5	4.2	4.2	3.7	3.3-4.2	4.7 (6.4-3.8)
Trickling Filter	12.5	16	11.5	13.5	16	13	11-16	12.8 (20.0-10.0)
Final Clarifier	18	19.5	20	34	35	>28	18-20	19.8 (33.0-9.5)
Refinery 6512								
Equalization	12.5[b]	18[b]	-	>56	42[b]	-	32-56	
Chem. Coag.	12.5[b]	28[b]	-	100	60	-	42-60	
				Stress				
Final Clarifier	100	100	-				100	
	No mortality or stress							
Refinery 0288								
Air Floatation	10.5[b]	50	-	34[b]	>56	-	24-56	
					Stress			
Final Clarifier	>48	100	-			-	100	
	No mortality or stress							
Refinery 6693								
API Separator	24	18	22.5	>32	41	22.5	18-24	
Final Clarifier	>41	100	-				100	
	No mortality or stress							

[a]Values in parenthesis are measured concentrations (at 24 hrs) in test containers bracketing the interpolated TL_{50} value.

[b]Low dissolved oxygen contributed to deaths in lower dilutions.

sludge systems of all refineries samples reduced toxicity. Final clarifier effluents from four refineries illicited no mortality or stress symptoms during the test period. The API separator effluent from refinery 2115 exerted a 24-hour TL_{50} of 4.2%-3.5% for three composite samples; corresponding values for the final clarifier effluent were 18%, 19.5%, and 20%. The major contributing toxic agent in this refinery is ammonia. Since the concentration of ammonia was essentially the same at both locations, the decrease in toxicity is most likely due to removal of contributing biodegradeable toxicants as well as a decrease in pH of the wastewater as it passed through the treatment system.

Observations were made of the behavioral patterns of distressed fish. These observations revealed the same four sypmtom patterns as those noted in Phase I of this study.

SECTION VI

DISCUSSION

PHASE I

Bioassay tests discussed herein were made using untreated process wastewaters from 17 selected oil refineries. Toxic characteristics of samples tested varied greatly between the 17 refineries. There is also a wide variation in toxicity of samples from different processes within an individual refinery. Results of the tests conducted and observations made during the tests for each individual refinery are discussed below.

Refinery 6992

Thirteen samples from this refinery were tested. Four of the samples exerted a 24-hour TL_{50} at dilutions tested: 0.042%, 0.043%, 0.05%, and 3.8%. Tests were extended over 48 hours with no change in the first three values and a reduction to 3.2% in the other test. The DO did not drop below 4.0 mg/1 during the first 24 hours in any of the tests; therefore, toxicity was due to wastewater constituents. Most of the toxicity exerted by these samples is due to high concentrations of constituents normally found in oil refinery wastewaters (ammonia, phenolic compounds, sulfide, cyanide) and was predictable from results of chemical analyses.

Symptoms exhibited by distressed fish were either those described in numbers 1 or 3 in Section V. These symptoms include those reported for the most common toxic constituents of oil refinery wastewaters.

Refinery 59991

The sample tested from this refinery exerted a 24-hour TL_{50} of 7.5%. The DO remained greater than 5.0 mg/1 throughout the test period;

therefore, toxicity was attributed to constituents in the wastewater. Symptoms of distressed fish were intermediate between numbers 1 and 4 in Section V, suggesting that the toxic agents were most likely ammonia and sulfide in combination. The TL_{50} value was predictable from results of chemical analysis.

Refinery 69991

This sample exerted a 24-hour TL_{50} of 13.5%. The DO remained greater than 3.5 mg/l throughout the test period; therefore, toxicity was attributed to wastewater constituents. Symptoms of dying fish were those listed in number 4 of Section V, suggesting that the toxic agents were most likely ammonia, sulfide, and cyanide in combination. The TL_{50} value was predictable from results of chemical analyses.

Refinery 693

Each of the three samples analyzed from this refinery exerted a 24-hour TL_{50} of <10% although toxicity in sample RW-3 was most likely due to low DO.

Sample RW-1 was much more toxic than predictable from results of chemical analyses. Dilutions were first set up from 10%-1%. Fish in all five dilutions became distressed immediately and were dead in less than two hours. Symptoms were those stated in number 2 of Section V. The sample had a definite creosote smell; therefore, the most likely toxic agents was one of the highly toxic phenolic compounds. Additional dilution series of 1%-0.1% and 0.1%-0.01% were set up to establish a 24-hour TL_{50}. In the 1%-0.1% series all test fish were in distress within four hours with the same symptoms. A 24-hour TL_{50} of 0.24% was established. Live fish in the remaining dilutions were in obvious distress. Fish in 0.1% dilution were removed at the end of test period and placed in clean aerated water. Symptoms appeared to be gone after six hours. Aeration was stopped and fish observed for 24 hours. All fish were alive and appeared healthy at the end of 24 hours.

Observation time was extended in 0.1%-0.01% dilution series to 36 hours. All fish were alive but in obvious distress at the end of 24 hours. Dissolved oxygen was greater than 4 mg/l at 24 hours but had dropped to 2.5 mg/l at 36 hours and tests were discontinued. The 36-hour TL_{50} of 0.05% may have been influenced by the low DO concentration. All live fish were in distress at the end of 36 hours, but fish at .018% and .010% did not become irritated as they did at higher dilutions.

Sample RW-2 exerted a 24-hour TL_{50} between 1.35%-2.40%. Insufficient sample was available to set up a 1.8% dilution. Symptoms were those listed in number 1 of Section V. The TL_{50} was predictable from the high ammonia values in the raw sample.

Refinery 69995

A 24-hour TL_{50} for these two sample could not be established from the dilutions tested and was reported as >10%. Some mortality and distress were noted at 10% dilution for sample RW-1; however, low DO definitely contributed to the mortality. Distress was observed prior to the DO drop. All fish in RW-2 dilutions were alive and healthy at end of test period.

Refinery 22

A 24-hour TL_{50} for these two samples could not be established from the dilutions tested and was reported as >18%. Fish at 18% for sample RW-2 were in distress at 12 hours, suggesting a 24-hour TL_{50} of 18%-32%. Dissolved oxygen was >4 mg/l at end of test.

Refinery 69994

Toxicity in sample RW-1 was predictable from results of chemical analyses. All fish in 10% dilution were dead within 30 minutes with symptoms described in number 1 of Section V, suggesting ammonia as

the major toxic agent. Eight fish were dead in 5.6% dilution within six hours; the two live fish remained in distress throughout the test. Dissolved oxygen was >4.0 mg/l at end of test.

Toxicity for sample RW-2 was mostly due to low pH (raw sample pH 0.9). All fish in 10% dilution were dead in five minutes with visable bleeding from gills (pH 2.1). All fish in 5.6% dilution were dead in 25 minutes and seven fish in 3.2% dilution were dead in 30 minutes (pH 2.6 and 3.25). One fish was alive in 3.2% dilution at end of test (pH 6.2 at 24 hours). All fish were alive and in no distress in 1% dilution (pH 7.1).

Since lack of sufficient sample prohibited a set-up at 1.8% dilution, the 24-hour TL_{50} was reported to be between 1.35%-2.50%.

Refinery 6993

No toxicity was exerted or distress observed at dilutions tested (TL_{50} >10%).

Refinery 595

No toxicity exerted or distress symptoms observed during test period at dilutions tested for sample RW-1 (TL_{50} >10%). Sample RW-2 exerted no toxicity during 24 hours (TL_{50} >18%). Fish in 18% dilution were in obvious distress at surface throughout the test and in 10% dilution after 12 hours, suggesting a 24-hour TL_{50} for 18%-32%. Dissolved oxygen was low at 24 hours.

Refinery 6995

A 24-hour TL_{50} was established for five of 10 samples tested. Three of the toxic samples, RW-3, RW-4, and RW-6, exerted toxicity greater than that predictable from results of chemical analyses. All live fish for these three samples were in distress at the end of 24 hours. Symptoms for all three samples were those listed in number 4 of Section V. Toxicity of these samples was most likely due to toxic hydrocarbon compounds. A plasticizer smell was noted.

Mortality for samples RW-1 and RW-5 was predictable from results of chemical analyses. Symptoms exhibited were those listed in numbers 1 and 4 of Section V. The most likely toxic agents were combinations of ammonia and sulfide in RW-1 and ammonia, sulfide, and phenolics in RW-5. Low DO may have contributed to mortality in 5.6% dilution for sample RW-1.

Fish in samples RW-2, RW-7, RW-9, and RW-10 exhibited no stress during the test period at the dilutions tested. Two fish died in samples RW-8 in 18% dilution and remaining fish were in obvious distress after 12 hours. Since DO remained >4.5 mg/1 throughout the test, a TL_{50} of between 18%-32% could be predicted for this sample.

Refinery 92

Samples RW-1 exerted no toxicity during the test period at the dilutions tested. Fish in 10% and 5.6% dilutions were in distress at surface at end of test due primarily to low DO.

Sample RW-2 exerted a TL_{50} of 2.4% for the test period with the DO remaining greater than 5.0 mg/1. Distress was noted in 1.8% dilution after 12 hours. Symptoms of distressed fish were those listed in number 1 in Section V. Toxicity in this sample was predictable from results of chemical analyses (phenol 144 mg/1; NH_3 85 mg/1).

Refinery 59

Although a TL_{50} could not be established at the dilutions tested (TL_{50} >10%), all fish exhibited an abnormal behavior after 12 hours. Dissolved oxygen remained adequate throughout the test.

Refinery 59993

No toxicity was exerted or distress symptoms exhibited at dilutions tested during the test period for these two samples (TL_{50} >10%).

Refinery 106

No toxicity was exerted during test period at dilutions tested. Fish in 18% and 10% dilutions exhibited distress symptoms at end of test period primarily due to low DO (TL_{50} >18%).

Refinery 56

No toxicity was exerted during the test period at the dilutions tested; however, fish at 10% and 5.6% dilutions exhibited distress symptoms after six hours (TL_{50} >10%).

Refinery 67

No toxicity was exerted or distress symptoms exhibited during test period at dilutions tested (TL_{50} >10%).

Refinery 6996

Three of the five samples tested exerted a 24-hour TL_{50} at dilutions tested. Sample RW-3 exerted toxicity much greater than indicated by results of chemical analyses.

No toxicity was exerted or distress symptoms exhibited in sample RW-1 at dilutions tested (TL_{50} >10%). Sample RW-2 exerted no toxicity at dilutions tested (TL_{50} >10%); however, fish in the 10% dilution were in distress at surface at 12 hours with DO >5.0 mg/l throughout the test period. A 24-hour TL_{50} of 10%-18% is indicated.

Sample RW-3 exhibited symptoms described in number 4 of Section V. Fish in 3.2% and 1.8% dilutions were in obvious distress at end of 24-hour period; no distress was observed in 1.0% dilution. Test was extended through 48 hours with some mortality in 3.2% and 1.8%; fish in 1.0% were not in distress at 48 hours. Dissolved oxygen remained sufficiently high throughout the 48-hour period to have no effect on results. The toxic agent is unknown but a hydrocarbon compound is suspected.

Fish in sample RW-4 exhibited symptoms described in number 3 of Section V, while those in sample RW-5 exhibited symptoms described in number 1. Dissolved oxygen remained >4.0 mg/l in both tests throughout the test period. Toxicity in both samples was predictable from results of chemical analyses (RW-4: ammonia 355 mg/l; RW-5: ammonia 3,500 mg/l, phenol 400 mg/l, sulfide 3,000 mg/l, cyanide 128 mg/l). With the ammonia value of 355 mg/l, RW-4 predicted to be more toxic than the bioassay indicated.

PHASE II

Bioassay tests discussed herein were conducted using samples from various treatment stages of activated sludge treatment systems at five selected oil refineries. Location of sample points in these refineries are noted in Figure 2. Toxic characteristics of the samples tested varied considerably between different oil refineries and between different treatment stages within individual refineries. Toxicity decreased at all selected refineries as the degree of treatment increased. Results of the tests conducted and observations made during the tests for each individual refinery are discussed below.

Refinery 9973

Effluent samples from the API separator and final clarifier were tested from the treatment system of this refinery. Although low DO after 12 hours prevented establishment of a reliable 24-hour TL_{50} for the separator effluent, some toxicity was exerted. Twelve-hour TL_{50}'s of 42%, 34%, and 41% for the three composites tested were obtained prior to the DO drop. A rerun of a composite of all samples using five fish in ten liters gave no mortality at 40%; therefore, the 24-hour TL_{50} for this sample is estimated to be approximately the same as the 12-hour value. Symptoms of dying fish were those listed in number 2 in Section V. Death was most likely due to the combined toxic action of constituents usually found in oil refinery wastewaters

(ammonia, phenolics, sulfides, cyanides). No mortality was exerted or distress symptoms observed in 24 hours in a 100% composite sample of the final clarifier effluent.

Refinery 2115

Samples from effluents of all three stages of treatment exerted a definite 24-hour TL_{50}. Three composite samples for each stage of treatment were tested with toxicity decreasing as the stage of treatment progressed from approximately 4% for the API separator effluent to approximately 20% for the final clarifier effluent. The high ammonia content at all stages of treatment was most likely the major contributing toxic agent, although symptoms of dying fish indicate some other toxic agent or agents were present. Stress symptoms were those listed in numbers 1 or 3 of Section V. Higher concentrations of ammonia seemed to be necessary for the same level of toxicity as the level of treatment progressed. A corresponding decrease in pH as treatment progresses most likely influenced ammonia toxicity in these tests since toxicity of ammonia increases with an increase in pH.[5, 10]

Refinery 6512

Tests were run on two composite samples of effluents from each of three stages of treatment. Effluent samples from the equalization and chemical coagulation units exhibited a demand that depleted the oxygen to a level where test results are considered unreliable. Five fish were used in ten liters of solution for the test of composite B. This did not decrease the effect of low DO enough to make the results reliable. Based on stress symptoms of test fish and early mortality during the tests, the 24-hour TL_{50} is estimated to be between 32%-56% for equalization unit samples and 42%-60% for chemical coagulation unit samples discounting effects of low DO. Stress symptoms were those listed in

number 4 of Section V. No mortality occurred or stress symptoms were observed during the test period for the final clarifier effluent sample in a 100% solution.

Refinery 0289

Tests were run on two composite samples for each of two stages of treatment. Low DO in composite A for the air flotation unit sample prevented establishment of a reliable TL_{50}. By using five fish in ten liters of solution for composite B, a TL_{50} of 50% was obtained. Since the final DO was 2.5 mg/l a range of 24%-56% is estimated for this sample. No mortality occurred or stress symptoms were observed in a 100% sample solution of the final clarifier effluent.

Refinery 6693

Tests were run on three composite samples of the API separator effluent and two composites of the final clarifier effluent. The 24-hour TL_{50} of approximately 21% (18%, 22.5%, and 24%) for the API separator effluent was reduced to where no mortality occurred or stress symptoms were observed in a 100% solution of the final clarifier effluent. Stress symptoms in the API tests were a combination of those listed in numbers 1 and 3 of Section V.

SECTION VII

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SECTION VIII

APPENDICES

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APPENDIX A

RAW DATA FOR TOXIC SAMPLES - PHASE I

Sample 6992:RW-2 TL_{50} - 0.042%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO[a] (mg/1)	Temp.[a] (° C)	pH Start	Sulfide[a] (mg/1)
0.1	10	0	7.0	23	8.6	1.2
0.056	10	2	6.3	23	8.6	1.1
0.032	10	8	4.3	23	8.3	0.9
0.018	10	9	-	23	8.4	-
0.010	10	10	-	23	8.6	-
Control	10	10	3.5	23	8.6	-

Sample 6992:RW-3 TL_{50} - 0.043

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO (mg/1)	Temp. (° C)	pH Start
0.1	10	0	8.6	22	-
0.056	10	1	-	-	-
0.032	10	10	4.3	22	8.6
0.018	10	9	-	-	-
0.010	10	9	-	23	-
Control	10	10	3.7	22	8.65

Sample 6992:RW-10 TL_{50} - 0.05%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO (mg/1)	Temp. (° C)	pH Start
0.1	10	0	5.8	23	8.7
0.056	10	4	5.2	24	-
0.032	10	9	2.2	22	-
0.018	10	8	-	-	-
0.010	10	9	-	-	-
Control	10	10	3.8	23	8.6

[a]DO, temperature, sulfide, ammonia, and phenol measurements were taken at 24 hours or at the death of all 10 fish.

Sample 6992:RW-11 TL_{50} - 3.8%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO[a] (mg/1)	Temp.[a] (° C)	pH Start
5.6	10	3	6.0	23	8.5
3.2	10	6	-	-	8.5
1.8	10	10	-	-	-
1.0	10	9	-	-	-
0.56	10	9	-	-	-
Control	10	10	3.7	23	8.7

Sample 59991:RW-1 TL_{50} - 7.5%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO (mg/1)	Temp. (° C)	pH Start
10	10	0	7.2	23.5	8.7
5.6	10	10	4.0	24	8.6
3.2	10	10	-	-	-
1.8	10	10	-	-	-
1.0	10	10	-	-	-
Control	10	10	4.6	24	8.6

Sample 69991:RW-1 TL_{50} - 13.5%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO (mg/1)	Temp. (° C)	pH Start
18	10	0	4.0	24.5	8.2
10	10	10	2.5	24	8.4
5.6	10	10	2.8	24	8.4
3.2	10	10	-	-	-
1.8	10	10	-	-	-
Control	10	10	4.0	24.5	8.3

[a]DO, temperature, sulfide, ammonia, and phenol measurements were taken at 24 hours or at the death of all 10 fish.

Sample 693:RW-1 TL_{50} - 0.23%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO[a] (mg/l)	Temp.[a] (° C)	pH Start	Phenol[a] (mg/l)
1.0	10	0	8.0	21.5	8.5	0.89
0.56	10	0	8.0	22	8.4	-
0.32	10	0	7.5	23.5	-	-
0.18	10	9	4.1	24	-	-
0.10	10	10	-	24	-	0.07
Control	10	10	3.7	24	-	-

Sample 693:RW-2 TL_{50} - 1.8%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO (mg/l)	Temp. (° C)	pH Start	Ammonia[a] (mg/l)
10	10	0	8.7	22.5	9.5	16.5
5.6	10	0	8.8	23	9.2	9.0
3.2	10	0	7.5	24	9.1	4.8
1.8	No set up	x	x	x	x	x
1.0	10	10	4.3	24	8.6	2.3
Control	10	10	4.6	24	-	-

Sample 693:RW-3 TL_{50} - 7.5%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO (mg/l)	Temp. (° C)	pH Start
10.0	10	0	0.5	25	8.4
5.6	10	10	4.6	25	-
3.2	10	10	-	-	-
1.8	10	10	-	-	-
1.0	10	10	-	-	-
Control	10	10	4.3	25	-

[a]DO, temperature, sulfide, ammonia, and phenol measurements were taken at 24 hours or at the death of all 10 fish.

Sample 69994:RW-1 TL_{50} - 4.5%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO[a] (mg/1)	Temp.[a] (° C)	pH Start	Ammonia[a] (mg/1)
10	10	0	8.5	23	9.0	12.0
5.6	10	2	5.0	25	-	6.5
3.2	10	10	4.1	23.5	-	4.0
1.8	10	10	-	-	-	-
1.0	10	10	-	-	-	-
Control	10	10	4.2	24	-	-

Sample 69994:RW-2 TL_{50} - 1.9%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO (mg/1)	Temp. (° C)	pH Start
10	10	0	7.8	22	2.1
5.6	10	0	-	-	2.6
3.2	10	1	7.2	24.5	3.25
1.8	No set up	x	x	x	x
1.0	10	10	4.5	24.5	7.2
Control	10	10	4.5	24	8.55

Sample 6995:RW-1 TL_{50} - 5.6%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO (mg/1)	Temp. (° C)	pH Start	Ammonia (mg/1)
10	10	0	7.5	23.5	9.0	9.0
5.6	10	7	1.6	25	8.7	5.4
3.2	10	9	4.1	24.5	-	3.3
1.8	10	10	-	-	-	-
1.0	10	10	-	-	-	-
Control	10	10	4.5	25	-	-

[a]DO, temperature, sulfide, ammonia, and phenol measurements were taken at 24 hours or at the death of all 10 fish.

Sample 6995:RW-3 TL_{50} - 13%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO[a] (mg/1)	Temp.[a] (° C)	pH Start
18	10	0	7.3	24	8.6
10	10	9	4.5	24	-
5.6	10	10	-	-	-
3.2	10	10	-	-	-
1.8	No set up	x	x	x	x
Control	10	10	4.7	24	-

Sample 6995:RW-4 TL_{50} - 13.5%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO (mg/1)	Temp. (° C)	pH Start
18	10	2	4.5	24	8.55
10	10	8	4.8	24	-
5.6	10	10	-	-	-
3.2	10	10	-	-	-
1.8	No set up	x	x	x	x
Control	10	10	4.0	24	-

Sample 6995:RW-5 TL_{50} - 4.2%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO (mg/1)	Temp. (° C)	pH Start
5.6	10	0	4.7	24	8.85
3.2	10	10	3.4	24	-
1.8	10	10	-	-	-
1.0	10	10	-	-	-
0.56	10	10	-	-	-
Control	10	10	2.6	23.5	-

[a]DO, temperature, sulfide, ammonia, and phenol measurements were taken at 24 hours or at the death of all 10 fish.

Sample 6995:RW-6 TL_{50} - 7.2%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO[a] (mg/l)	Temp.[a] (° C)	pH Start
18	10	0	7.0	22	-
10	10	0	7.0	23.5	8.4
5.6	10	9	5.6	23	8.4
3.2	10	10	-	-	-
1.8	No set up	x	x	x	x
Control	10	10	-	24	-

Sample 92:RW-2 TL_{50} - 2.4%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO (mg/l)	Temp. (° C)	pH Start	Ammonia[a] (mg/l)	Phenol[a] (mg/l)
10	10	0	6.5	22.0	9.2	14.2	11.7
5.6	10	0	5.7	23.0	9.1	8.1	6.9
3.2	10	0	5.3	23.5	9.0	4.6	4.0
1.8	10	10	2.5	23.5	-	3.3	-
1.0	10	10	-	-	-	2.0	-
Control	10	10	4.6	23.5	-	0.7	-

Sample 6996:RW-3 TL_{50} - 4.2%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO (mg/l)	Temp. (° C)	pH Start
10	10	0	6.9	22.5	7.7
5.6	10	0	7.0	22	-
3.2	10	10	4.8	21.5	-
1.8	10	10	5.6	21.5	-
1.0	10	10	-	-	-
Control	10	10	6.0	21.5	8.4

[a]DO, temperature, sulfide, ammonia, and phenol measurements were taken at 24 hours or at the death of all 10 fish.

Sample 6996:RW-4 TL_{50} - 4.2%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO[a] (mg/l)	Temp.[a] (° C)	pH Start
5.6	10	0	6.5	23	8.25
3.2	10	10	3.8	22	8.50
1.8	10	9	-	-	8.50
1.0	10	10	-	-	-
0.56	10	10	-	-	-
Control	10	10	3.7	22	8.40

Sample 6996:RW-5 TL_{50} - 0.05%

Conc. of Waste (% by vol.)	No. of Test Animals	No. Alive at 24 hrs	DO (mg/l)	Temp. (° C)	pH Start
0.1	10	0	7.3	22.5	8.65
0.056	10	4	5.6	22	8.45
0.032	10	10	0	23	-
0.018	10	9	-	-	-
0.010	10	10	-	-	-
Control	10	10	4.4	22.5	8.45

[a]DO, temperature, sulfide, ammonia, and phenol measurements were taken at 24 hours or at the death of all 10 fish.

APPENDIX B

RAW DATA - PHASE II

SAMPLE REFINERY 9973: API SEPARATOR EFFLUENT

Composite A 12-hour TL_{50} - 42%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO[a] (mg/l) 12 hr	DO[a] (mg/l) 24 hr	Temp.[b] (° C)	pH Start	Ammonia (mg/l) Start
56	10	3	0	3.9	3.8	22	8.9	5.1
32	10	10	4	4.0	0.5	21.5	8.8	3.1
18	10	10	10	4.0	1.2	21.5	-	2.0
10	10	10	10	-	1.3	-	-	-
5.6	10	10	10	-	-	-	-	-
Control	10	10	10	5.2	3.1	21.5	8.7	-

Composite B 12-hour TL_{50} - 34%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
48	10	0	0	3.8	-	22	8.6
24	10	10	2	2.0	1.0	22	8.5
12	10	10	10	-	1.5	22	-
6	10	10	10	-	1.5	22	-
3	10	10	10	-	-	-	-
Control	10	10	10	4.8	2.5	22	8.4

Composite C 12-hour TL_{50} - 41%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
48	10	0	0	3.6	0.5	22	8.9
36	10	10	0	3.2	0.6	22	8.8
25	10	10	2	3.5	1.0	22	8.7
16.8	10	10	3	-	1.2	22	8.7
12.6	10	10	10	-	1.3	22	8.6
Control	10	10	10	4.9	3.1	22	8.6

[a]DO readings at 12 hours, 24 hours, or at death of all 10 fish.

[b]Temperature readings at 24 hours or at death of all 10 fish.

SAMPLE REFINERY 9973: FINAL CLARIFIER EFFLUENT

Composite A 24-hour TL_{50} - >56%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO[a] (mg/l) 12 hr	DO[a] (mg/l) 24 hr	Temp.[b] (° C)	pH Start	Ammonia (mg/l) Start
56	10	10	10	6.0	3.7	22	8.3	7.0
32	10	10	10	-	-	-	-	4.0
18	10	10	10	-	-	-	-	-
10	10	10	10	-	-	-	-	-
5.6	10	10	10	-	-	-	-	-
Control	10	10	10	5.1	3.0	22	8.2	-

Composite B 24-hour TL_{50} - >88%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
88	10	10	10	6.1	3.1	22.5	8.0
44	10	10	10	5.5	2.7	22	8.3
22	10	10	10	-	-	-	-
11	10	10	10	-	-	-	-
Control	10	10	10	3.8	1.5	22.5	8.4

Composite C 24-hour TL_{50} - 100% (no mortality or observed stress)

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
100	10	10	10	7.2	4.0	22	7.6
50	10	10	10	6.2	3.5	22	8.3
25	10	10	10	-	-	-	-
12.5	10	10	10	-	-	-	-
6.25	10	10	10	-	-	-	-
Control	10	10	10	4.0	2.0	22	8.6

[a] DO readings at 12 hours, 24 hours, or at death of all 10 fish.

[b] Temperature readings at 24 hours or at death of all 10 fish.

SAMPLE REFINERY 2115: API SEPARATOR EFFLUENT

Composite A 24-hour TL_{50} - 4.2%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO[a] (mg/l) 12 hr	DO[a] (mg/l) 24 hr	Temp.[b] (° C)	pH Start	Ammonia (mg/l) Start
10	10	0	0	7.6	-	21	9.4	11.1
5.6	10	0	0	7.7	-	21.5	9.0	6.4
3.2	10	10	10	5.7	2.7	22	8.9	3.8
1.8	10	10	10	-	-	-	8.6	2.25
1.0	10	10	10	-	-	-	-	1.1
Control	10	10	10	5.2	3.0	22	8.4	0.2

Composite B 24-hour TL_{50} - 4.2%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
10	10	0	0	7.8	-	21	9.3
5.6	10	0	0	7.6	-	21.5	9.1
3.2	10	10	10	4.8	2.5	22	8.8
1.8	10	10	10	-	-	-	-
1.0	10	10	10	-	-	-	-
Control	10	10	10	4.5	2.5	22	8.6

Composite C 24-hour TL_{50} - 3.5%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
7.5	10	0	0	8.1	-	20	9.3
5.6	10	0	0	8.1	-	20	9.1
4.2	10	0	0	7.5	-	22	9.0
3.2	10	10	8	5.1	2.2	22	8.8
2.4	10	10	10	5.5	1.7	22	8.7
Control	10	10	10	4.5	1.7	22	8.6

[a] DO readings at 12 hours, 24 hours, or at death of all 10 fish.

[b] Temperature readings at 24 hours or at death of all 10 fish.

SAMPLE REFINERY 2115: TRICKLING FILTER EFFLUENT

Composite A 24-hour TL_{50} - 12.5%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO[a] (mg/l) 12 hr	DO[a] (mg/l) 24 hr	Temp.[b] (° C)	pH Start	Ammonia (mg/l) Start
32	10	0	0	7.6	-	18	8.8	33.0
18	10	0	0	-	-	-	-	20.0
10	10	10	8	5.3	2.0	22	8.6	10.0
5.6	10	10	10	5.3	2.5	22	8.6	6.2
3.2	10	10	10	-	-	-	-	-
Control	10	10	10	5.8	2.6	22	8.7	-

Composite B 24-hour TL_{50} - 16%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
24	10	0	0	7.0	-	20	8.4
12	10	10	10	4.7	1.5	22	8.3
6	10	10	10	5.3	3.0	22	8.3
3	10	10	10	-	-	-	-
1.5	10	10	10	-	-	-	-
Control	10	10	10	4.7	2.5	22	8.5

Composite C 24-hour TL_{50} - 11.5%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
24	10	0	0	8.0	-	18	8.6
18	10	0	0	6.9	-	21	8.6
13.5	10	4	0	5.9	5.5	22	-
10.0	10	10	10	5.8	3.3	22	-
7.5	10	10	10	-	3.6	22	-
Control	10	10	10	5.7	4.0	22	8.6

[a]DO readings at 12 hours, 24 hours, or at death of all 10 fish.

[b]Temperature readings at 24 hours or at death of all 10 fish.

SAMPLE REFINERY 2115: FINAL CLARIFIER EFFLUENT

Composite A 24-hour TL_{50} - 18%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO[a] (mg/1) 12 hr	DO[a] (mg/1) 24 hr	Temp.[b] (° C)	pH Start	Ammonia (mg/1) Start
32	10	0	0	6.0	-	22	8.5	33.0
18	10	10	5	5.6	2.1	22	-	19.8
10	10	10	10	5.8	2.2	22	-	9.5
5.6	10	10	10	-	-	-	-	5.8
3.2	10	10	10	-	-	-	-	-
Control	10	10	10	6.3	3.1	22	8.7	-

Composite B 24-hour TL_{50} - 19.5%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/1) 12 hr	DO (mg/1) 24 hr	Temp. (° C)	pH Start
48	10	1	0	5.3	5.2	22	8.2
24	10	10	3	5.1	2.5	22	8.4
12	10	10	10	-	3.2	22	-
6	10	10	10	-	-	-	-
3	10	10	10	-	-	-	-
Control	10	10	10	5.0	3.1	22	8.6

Composite C 24-hour TL_{50} - 20%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/1) 12 hr	DO (mg/1) 24 hr	Temp. (° C)	pH Start
28	10	10	1	5.2	4.0	22.5	8.2
21	10	10	4	5.3	3.0	22.5	8.4
15.5	10	10	10	5.5	2.8	22.5	-
11.5	10	10	10	-	-	-	-
8.7	10	10	10	-	-	-	-
Control	10	10	10	5.5	4.0	22.5	8.5

[a]DO readings at 12 hours, 24 hours, or at death of all 10 fish.

[b]Temperature readings at 24 hours or at death of all 10 fish.

SAMPLE REFINERY 6512: EQUALIZATION UNIT EFFLUENT

Composite A Estimated 24-hour TL_{50} - 32%-56%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO[a] (mg/l) 12 hr	DO[a] (mg/l) 24 hr	Temp.[b] (° C)	pH Start
56	10	10	3	2.6	0.4	22.5	8.1
32	10	10	2	2.7	0.5	22.5	8.3
18	10	10	1	1.6	0.5	22.5	8.5
10	10	10	8	-	0.8	23	-
5.6	10	10	10	-	1.0	23	-
Control	10	10	10	2.5	1.1	23	8.6

Composite B Estimated 24-hour TL_{50} - 32%-56%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
56	5	0	0	1.0	-	22.5	8.0
32	5	5	0	2.2	1.0	23.5	8.3
18	No set up	x	x	x	x	x	x
10	5	5	5	4.2	2.1	23.5	8.6
5.6	5	5	5	5.5	3.0	23.5	-
Control	5	5	5	5.5	3.3	23.5	8.7

[a]DO readings at 12 hours, 24 hours, or at death of all 10 (5) fish.

[b]Temperature readings at 24 hours or at death of all 10 (5) fish.

SAMPLE REFINERY 6512: CHEMICAL COAGULATION UNIT EFFLUENT

Composite A Estimated 24-hour TL_{50} - 42%-60%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO[a] (mg/l) 12 hr	DO[a] (mg/l) 24 hr	Temp.[b] (° C)	pH Start
100	10	10	1	2.2	0.3	22.5	7.6
56	10	10	0	2.0	0.3	22.5	8.0
32	10	10	2	2.0	0.3	22.5	8.4
18	10	10	2	-	0.5	22.5	-
10	10	10	7	-	0.8	22.5	-
Control	10	10	10	2.7	1.1	23	8.7

Composite B Estimated 24-hour TL_{50} - 42%-60%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
67	5	2	0	2.8	0.5	22.5	7.7
40	5	4	0	3.2	0.6	22.5	8.0
20	5	5	5	4.4	1.5	23	-
10	5	5	5	-	-	-	-
5	5	5	5	-	-	-	-
Control	5	5	5	6.4	5.5	23	-

[a]DO readings at 12 hours, 24 hours, or at death of all 10 (5) fish.

[b]Temperature readings at 24 hours or at death of all 10 (5) fish.

SAMPLE REFINERY 6512: FINAL CLARIFIER EFFLUENT

Composite A 24-hour TL_{50} - 100% (no mortality or observed stress)

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO[a] (mg/l) 12 hr	DO[a] (mg/l) 24 hr	Temp.[b] (° C)	pH Start
100	10	10	10	6.2	2.4	22	7.4
56	10	10	10	5.5	2.5	22	8.3
32	10	10	10	-	2.7	22	-
18	10	10	10	-	-	-	-
10	10	10	10	-	-	-	-
Control	10	10	10	2.2	1.2	22.5	8.7

Composite B 24-hour TL_{50} - 100% (observed stree and 40% mortality)

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
100	5	5	5	6.5	3.7	23	7.4
50	5	5	5	6.2	3.5	23	7.9
25	5	5	5	6.0	4.0	23	-
12.5	5	5	5	-	-	-	-
Control	5	5	5	6.3	4.5	23	-

[a]DO readings at 12 hours, 24 hours, or at death of all 10 (5) fish.

[b]Temperature readings at 24 hours or at death of all 10 (5) fish.

SAMPLE REFINERY 0289: AIR FLOATATION

Composite A Estimated 24-hour TL_{50} - 32%-48%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO[a] (mg/l) 12 hr	DO[a] (mg/l) 24 hr	Temp.[b] (° C)	pH Start
48	10	0	0	1.2	-	23	8.4
24	10	10	2	2.2	0.9	23	8.6
12	10	10	4	1.5	0.8	23	8.7
6	10	10	10	-	1.1	23	-
3	10	10	10	-	1.1	23	-
Control	10	10	9	1.2	1.1	23	8.7

Composite B Estimated 24-hour TL_{50} - 50%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
56	5	5	2	4.2	2.0	23	8.0
32	5	5	5	4.7	2.2	23	8.4
18	5	5	5	5.0	2.9	23	8.5
10	5	5	5	-	-	-	-
5.6	5	5	5	-	-	-	-
Control	5	5	5	5.3	3.8	23	8.7

[a]DO readings at 12 hours, 24 hours, or at death of all 10 (5) fish.

[b]Temperature readings at 24 hours or at death of all 10 (5) fish.

SAMPLE REFINERY 0289: FINAL CLARIFIER EFFLUENT

Composite A 24-hour TL_{50} - >48%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO[a] (mg/l) 12 hr	DO[a] (mg/l) 24 hr	Temp.[b] (° C)	pH Start
48	10	10	9	3.4	1.2	23	8.6
24	10	10	9	2.5	1.2	23	8.7
12	10	10	10	2.5	1.4	23	-
6	10	10	10	-	-	-	-
Control	10	10	10	2.7	1.9	23.5	8.7

Composite B 24-hour TL_{50} - 100% (no mortality or observed stress)

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
100	5	5	5	7.0	5.1	23	7.7
50	5	5	5	6.6	5.5	23	8.1
25	5	5	5	6.3	4.5	23	-
12.5	5	5	5	-	-	-	-
Control	5	5	5	6.3	4.6	23	-

[a] DO readings at 12 hours, 24 hours, or at death of all 10 (5) fish.

[b] Temperature readings at 24 hours or at death of all 10 (5) fish.

SAMPLE REFINERY 6693: API SEPARATOR EFFLUENT

Composite A 24-hour TL_{50} - 24%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO[a] (mg/l) 12 hr	DO[a] (mg/l) 24 hr	Temp.[b] (° C)	pH Start
32	10	9	0	5.9	4.2	24	8.8
18	10	10	10	6.4	4.8	24	8.5
10	10	10	10	-	-	-	-
5.6	10	10	10	-	-	-	-
3.2	10	10	10	-	-	-	-
Control	10	10	10	6.4	4.1	24	8.4

Composite B 24-hour TL_{50} - 18%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
56	10	0	0	8.5	-	22.5	8.9
32	10	1	0	6.6	6.6	24	8.8
18	10	10	5	-	2.0	-	-
10	10	10	9	-	-	-	-
5.6	10	10	10	-	-	-	-
Control	10	10	10	5.8	3.7	24.5	8.8

Composite C 24-hour TL_{50} - 22.5%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
56	10	0	0	7.7	-	20	9.0
32	10	0	0	7.0	-	23	8.9
18	10	8	8	6.0	2.8	23	8.8
10	10	10	10	-	-	-	-
5.6	10	10	10	-	-	-	-
Control	10	10	10	6.4	4.8	23	-

[a]DO readings at 12 hours, 24 hours, or at death of all 10 fish.

[b]Temperature readings at 24 hours or at death of all 10 fish.

SAMPLE REFINERY 6693: FINAL CLARIFIER EFFLUENT

Composite A 24-hour TL_{50} - >41%

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO[a] (mg/l) 12 hr	DO[a] (mg/l) 24 hr	Temp.[b] (° C)	pH Start
41	10	10	10	7.6	5.9	24	8.1
32	10	10	10	-	-	-	-
18	10	10	10	-	-	-	-
10	10	10	10	-	-	-	-
Control	10	10	10	7.5	6.6	24	8.7

Composite B 24-hour TL_{50} - 100% (no mortality or distress observed)

Conc. of Waste (% by vol)	No. of Test Animals	No. alive at 12 hrs	No. alive at 24 hrs	DO (mg/l) 12 hr	DO (mg/l) 24 hr	Temp. (° C)	pH Start
100	5	5	5	7.0	3.8	24.5	7.7
50	5	5	5	5.7	-	-	8.4
25	5	5	5	-	-	-	-
12.5	5	5	5	-	-	-	-
Control	5	5	5	4.4	3.0	25	8.7

[a]DO readings at 12 hours, 24 hours, or at death of all 10 (5) fish.

[b]Temperature readings at 24 hours or at death of all 10 (5) fish.

APPENDIX C

ANALYTICAL DATA - PHASE I AND PHASE II

RESULTS OF CHEMICAL ANALYSES OF PHASE I SAMPLES
COLLECTED CONCURRENT WITH BIOASSAY SAMPLES

Sample No.[a]	Chemical Parameter			
	Ammonia (mg/l)	Phenolics (mg/l)	Sulfide (mg/l)	Cyanide (mg/l)
6992 RW-2	3575	525	3975	0.6
6992 RW-3	225	14,500	37	17.0
6992 RW-10	3350	500	3846	1.4
6992 RW-11	95	1.4	129	0.34
5991 RW-1	118	7.6	84	0.74
69991 RW-1	40	2.9	14	1.4
693 RW-1	95	64	51	0.22
693 RW-2	199	0.52	11	0.14
69994 RW-1	125	4.5	19	0.38
6995 RW-1	81	5.4	110	0.03
6995 RW-3	1.3	0.52	0.4	<0.03
6995 RW-4	1.5	0.52	0.9	<0.03
6995 RW-5	120	62	78	0.1
6995 RW-6	0.85	0.38	0.2	<0.03
92 RW-2	85	140	3.40	0.08
6996 RW-3	5.2	3.1	1.4	<0.03
6996 RW-4	350	<0.01	1.0	<0.03
6996 RW-5	2700	410	3000	122

[a]Samples for which definite TL_{50} was established.

RESULTS OF CHEMICAL ANALYSES OF PHASE II SAMPLES COLLECTED CONCURRENT WITH BIOASSAY SAMPLES[a]

Sample Location	Chemical Parameter			
	Ammonia (mg/1)	Phenolics (mg/1)	Sulfide (mg/1)	Cyanide (mg/1)
Refinery 9973				
API Separator	9.25	11.0	2.88	0.135
Final Clarifier	6.4	0.01	0.15	0.10
Refinery 2115				
API Separator	127	10.45	24.3	1.50
Trickling Filter	108.5	0.99	0.75	0.245
Final Clarifier	106	0.035	0.275	0.07
Refinery 6512				
Equal. Chamber	6.9	0.61	1.0	0.22
Chem. Coag. Unit	6.75	0.405	1.5	0.20
Final Clarifier	0.375	0.01	0.425	0.14
Refinery 0288				
Air Float. Unit	12.0	0.02	0.7	0.06
Final Clarifier	11.25	0.01	0.5	0.02
Refinery 6693				
API Separator	28.4	3.4	32.5	0.29
Final Clarifier	26.25	0.01	0.2	0.185

[a]The median value from results of analyses of samples collected on 14 consecutive days at each location is presented.